

CLAIMS

1. A modified nucleotide or nucleoside molecule comprising a purine or pyrimidine base and a ribose or deoxyribose sugar moiety having a removable 3'-OH blocking group covalently attached thereto, such that the 3' carbon atom has attached a group of the structure



- wherein Z is any of $-C(R')_2-O-R''$, $-C(R')_2-N(R'')_2$, $-C(R')_2-N(H)R''$, $-C(R')_2-S-R''$ and $-C(R')_2-F$,

wherein each R'' is or is part of a removable protecting group;

- each R' is independently a hydrogen atom, an alkyl, substituted alkyl, arylalkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocyclic, acyl, cyano, alkoxy, aryloxy, heteroaryloxy or amido group, or a detectable label attached through a linking group; or $(R')_2$ represents an alkylidene group of formula $=C(R''')_2$ wherein each R''' may be the same or different and is selected from the group comprising hydrogen and halogen atoms and alkyl groups; and

- wherein said molecule may be reacted to yield an intermediate in which each R'' is exchanged for H or, where Z is $-C(R')_2-F$, the F is exchanged for OH, SH or NH_2 , preferably OH, which intermediate dissociates under aqueous conditions to afford a molecule with a free 3'OH;

- with the proviso that where Z is $-C(R')_2-S-R''$, both R' groups are not H.

2. A molecule according to claim 1 wherein R' is an alkyl or substituted alkyl.

- 118 -

3. A molecule according to claim 1 or claim 2
wherein -Z is of formula $-C(R')-N_3$.
4. A molecule according to any one of claims 1 to 3
5 wherein Z is an azidomethyl group.
5. A molecule according to claim 1 or claim 2
wherein R" is a benzyl or substituted benzyl group.
- 10 6. A molecule according to any preceding claim
wherein said base is linked to a detectable label via
a cleavable linker or a non-cleavable linker.
7. A molecule according to claim 6 wherein said
15 linker is cleavable.
8. A molecule according to any one of claims 1 to 5
wherein a detectable label is linked to the molecule
through the blocking group by a cleavable or non-
20 cleavable linker.
9. A molecule according to any one of claims 6 to 8
wherein said detectable label is a fluorophore.
- 25 10. A molecule according to any one of claims 6 to 9
wherein said linker is acid labile, photolabile or
contains a disulfide linkage.
11. A modified nucleotide molecule as claimed in any
30 one of claims 1 to 10 which comprises one or more ^{32}P
atoms in its phosphate portion.
12. A nucleoside, nucleotide or polynucleotide

- 119 -

molecule of formula PN-O-allyl, wherein PN is said nucleoside or nucleotide or is a 3'terminal nucleotide of said polynucleotide; and said nucleoside or nucleotide further comprises in addition to the allyl blocking group a detectable label linked to the base thereof by a cleavable or non-cleavable linker.

13. A molecule according to claim 12 wherein said linker is cleavable.

14. A molecule according to claim 12 or claim 13 wherein said detectable label is a fluorophore.

15. A molecule according to any one of claims 12 to 14 wherein said linker is acid labile, photolabile or contains a disulfide linkage.

16. A method of converting a compound of formula R-O-allyl, R₂N(allyl), RNH(allyl), RN(allyl)₂ or R-S-allyl to a corresponding compound in which the allyl group is removed and replaced by hydrogen, said method comprising the steps of reacting a compound of formula R-O-allyl, R₂N(allyl), RNH(allyl), RN(allyl)₂ or R-S-allyl in aqueous solution with a transition metal comprising a transition metal and one or more ligands selected from the group comprising water-soluble phosphine and water-soluble nitrogen-containing phosphine ligands, wherein the or each R is a water-soluble biological molecule.

17. The method of claim 16 wherein said compound is of formula R-O-allyl.

- 120 -

18. The method of claim 16 or claim 17 wherein said R is part of a nucleoside, a nucleotide or a polynucleotide molecule.

5 19. The method of claim 18 wherein said nucleoside, nucleotide or polynucleotide further comprises a detectable label linked to the base thereof by a cleavable or non-cleavable linker.

10 20. A molecule according to claim 19 wherein said linker is cleavable.

21. The method of claim 19 or claim 20, wherein said detectable label is a fluorophore.

15

22. The method of any one of claims 19 to 21 wherein said linker is acid labile, photolabile or contains a disulfide linkage.

20 23. The method of any one of claims 19 to 22 wherein said allyl group and said label are removed in a single step.

25 24. The method of any one of claims 16 to 23 wherein said transition metal is selected from the group comprising platinum, palladium, rhodium, ruthenium, osmium and iridium.

30 25. The method of any one of claims 16 to 24 wherein said transition metal is palladium.

26. The method of any one of claims 16 to 25 wherein said group of ligands comprise derivatised triaryl

- 121 -

phosphine ligands or derivatised trialkyl phosphine ligands.

27. The method of any one of claims 16 to 26 wherein
5 said group of ligands are derivatised with one or more functionalities selected from the group comprising amino, hydroxyl, carboxyl and sulfonate groups.

28. The method of any one of claims 16 to 27 wherein
10 the group of ligands comprises 3,3',3"-phosphinidynetris (benzenesulfonic acid) and tris(2-carboxyethyl)phosphines and their salts.

29. A method of controlling the incorporation of a
15 nucleotide as defined in any one of claims 6 to 10 or as defined in any one of claims 12 to 15 and complementary to a second nucleotide in a target single-stranded polynucleotide in a synthesis or sequencing reaction comprising incorporating into the
20 growing complementary polynucleotide said nucleotide, the incorporation of said nucleotide preventing or blocking introduction of subsequent nucleoside or nucleotide molecules into said growing complementary polynucleotide.

25

30. The method of claim 29, wherein the incorporation of said nucleotide is accomplished by a terminal transferase or polymerase or a reverse transcriptase.

30 31. The method of claim 30 wherein the polymerase is a *Thermococcus* sp.

32. The method of claim 31 wherein the *Thermococcus*

- 122 -

sp is 9°N or a single mutant or double mutant thereof.

33. The method of claim 32 wherein the double mutant is -Y409V A485L.

5

34. A method for determining the sequence of a target single-stranded polynucleotide, comprising monitoring the sequential incorporation of complementary nucleotides, wherein at least one incorporation is of
10 a nucleotide as defined in any one of claims 6 to 10 or as defined in any one of claims 12 to 15 and wherein the identity of the nucleotide incorporated is determined by detecting the label linked to the base, and the blocking group and said label are removed
15 prior to introduction of the next complementary nucleotide.

35. The method of claim 34 wherein the label of the nucleotide and the blocking group are removed in a
20 single chemical treatment step.

36. A method for determining the sequence of a target single-stranded polynucleotide, comprising:

(a) providing a plurality of different
25 nucleotides wherein said plurality of different nucleotides are either as defined in any one of claims 6 to 10 or as defined in any one of claims 12 to 15 and wherein the detectable label linked to each type of nucleotide can be distinguished upon detection from
30 the detectable label used for other types of nucleotides;

(b) incorporating the nucleotide into the complement of the target single-stranded

- 123 -

polynucleotide;

(c) detecting the label of the nucleotide of (b), thereby determining the type of nucleotide incorporated;

5 (d) removing the label of the nucleotide of (b) and the blocking group; and

(e) optionally repeating steps (b)-(d) one or more times;

thereby determining the sequence of a target
10 single-stranded polynucleotide.

37. The method of claim 36 wherein said incorporating step is accomplished by a *Thermococcus* sp.

15

38. The method of claim 37 wherein the *Thermococcus* sp is 9°N or a single mutant or double mutant thereof.

20

39. The method of claim 38 wherein the double mutant is -Y409V A485L.

40. The method of any one of claims 36 to 39 wherein the label of the nucleotide and the blocking group are removed in a single chemical treatment step.

25

41. A method according to any one of claims 36 to 40, wherein each of the nucleotides are brought into contact with the target sequentially, with removal of non-incorporated nucleotides prior to addition of the next nucleotide, and wherein detection and removal of the label and the blocking group is carried out either
30 after addition of each nucleotide, or after addition of all four nucleotides.

- 124 -

42. The method according to any one of claims 36 to 40, wherein each of the nucleotides are brought into contact with the target together simultaneously, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label and the blocking group.

43. The method according to any one of claims 36 to 40, comprising a first step and a second step, wherein in the first step, a first composition comprising two of the four nucleotides is brought into contact with the target and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label, and wherein in the second step, a second composition comprising the two nucleotides not included in the first composition is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label and blocking group, and wherein the first and second steps are optionally repeated one or more times.

44. The method according to any one of claims 36 to 40, comprising a first step and a second step, wherein in the first step, a composition comprising one of the four nucleotides is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label and blocking group and wherein in the second step, a second composition comprising the three nucleotides not included in the first composition is brought into contact with the target, and non-

- 125 -

incorporated nucleotides are removed prior to detection and subsequent to removal of the label and blocking group and wherein the first steps and the second step are optionally repeated one or more times.

5

45. The method according to any one of claims 36 to 40, comprising a first step and a second step, wherein in the first step, a first composition comprising three of the four nucleotides is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label and blocking group and wherein in the second step, a composition comprising the nucleotide not included in the first composition is brought into contact with the target, and non-incorporated nucleotides are removed prior to detection and subsequent to removal of the label and blocking group and wherein the first steps and the second step are optionally repeated one or more times.

20

46. A kit, comprising:

(a) a plurality of different nucleotides wherein said plurality of different nucleotides are either as defined in any one of claims 6 to 10 or as defined in any one of claims 12 to 15; and

(b) packaging materials therefor.

47. A kit according to claim 46, wherein the detectable label in each nucleotide can be distinguished upon detection from the detectable label used for any of the other three types of nucleotide.

30

48. The kit of claim 46 or 47, further comprising an

- 126 -

enzyme and buffers appropriate for the action of the enzyme.

49. Use of a nucleotide as defined in any one of
5 claims 1 to 15 in a Sanger or a Sanger-type sequencing method.

50. A method of using a nucleotide of claim 1 wherein
said method includes a Sanger or Sanger-type
10 sequencing method.